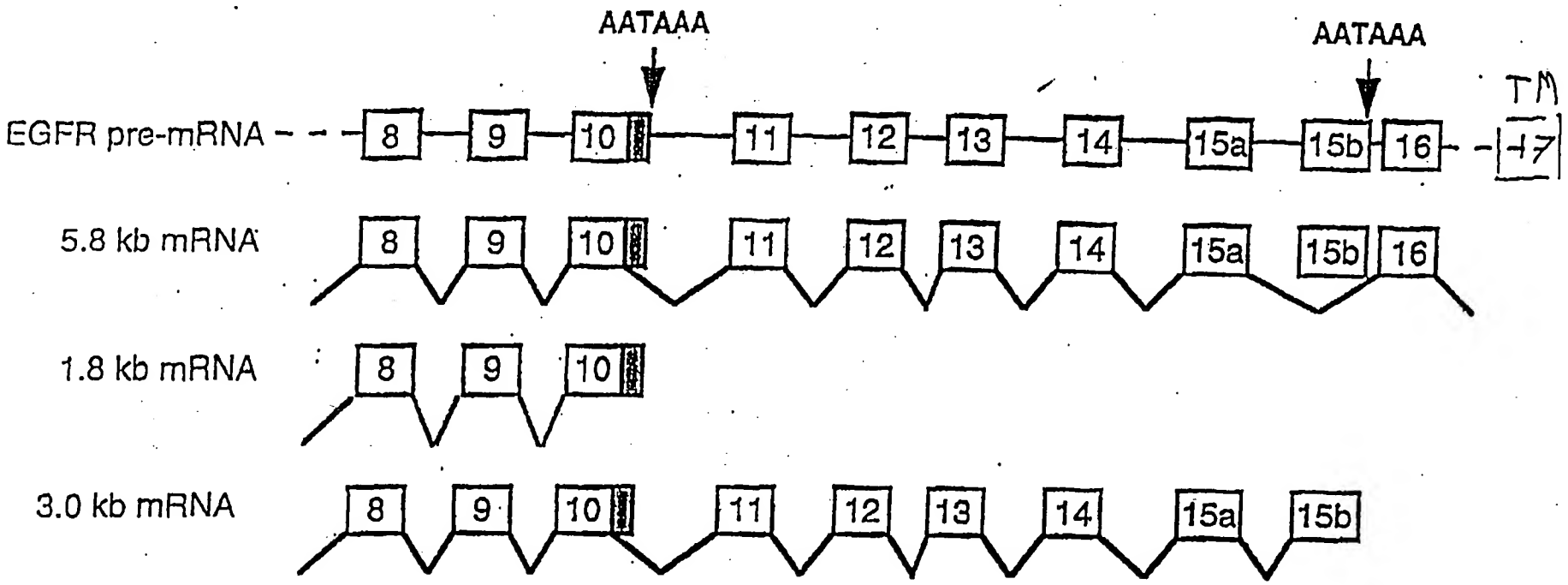
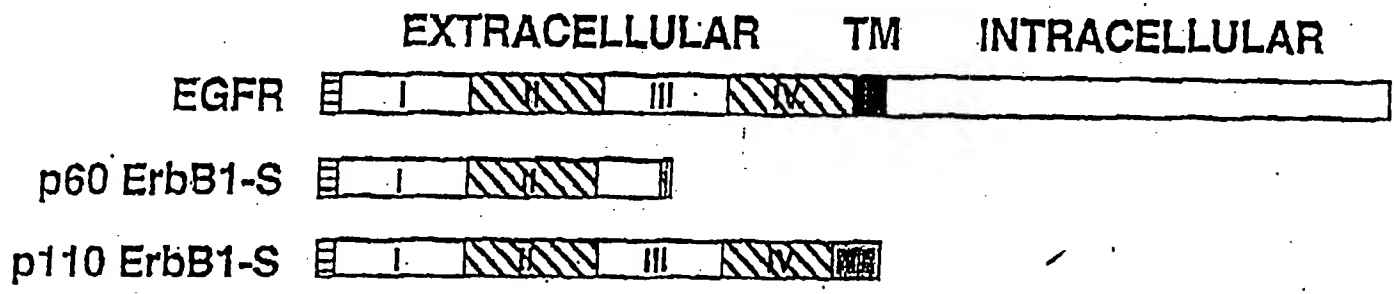


FIGURE 1



p60 ErbB1-S

- encoded by 1.8 kb transcript

  mature product = 60 kDa
- Contains 381 amino acids
  - unique a.a: Leu and Ser

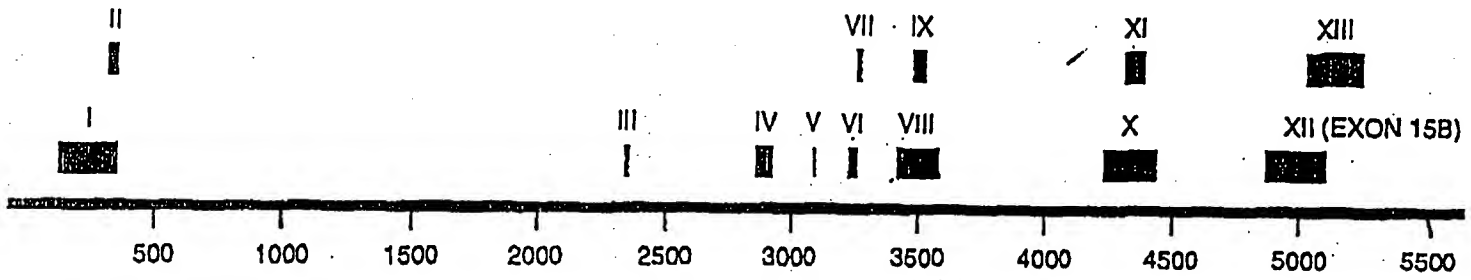
  Calculated mw = 45 kDa
  - minus signal peptide = 42 kDa

p110 ErbB1-S

- encoded by 3.0 kb transcript
- mature product = 110 kDa
- Contains 681 amino acids
  - 78 unique a.a
- Calculated mw = 77 kDa
  - minus signal peptide = 75 kDa

FIGURE 2

Alternative Exons Located In Human EGFR Intron 15



Alternative Exons Located In Human EGFR Intron 16

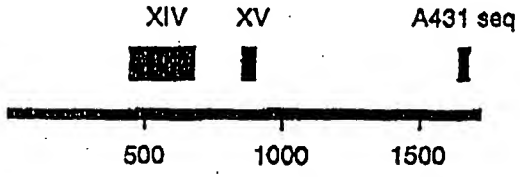


FIGURE 3

Seq ID	Alternative Exons (coding sequence only)	IVS #	Amino Acids	Translated Peptides
Exon 15	cag GGACCAGACAACTGTATCCAGTGTGCCCACTACATTGACGGCCCCCACTG CGTCAAGACCTGCCCGGCAGGAGTCATGGGAGAAAACAACACCCTGGTCTGGAAG TACGCAGACGCCGGCCATGTGTGCCACCTGTGCCATCCAAACTGCACCTACGG	NA	53	GPDNCIQCAHYIDGPHCVKTCP AGVMGENNTLVWKYADAGHVCH LCHPNCTYG
I	cag CCATGCCAGTAGCAACTTGCTTGTGAGCAGGCCTCAGTGCAGTGGGAATG ACTCTGCCATGCACCGTGTCCCCGGCCGGGCCTGTGTTGTGCAATGCTGCACAT CACAACAGGAGGGTAGGGGGACAAAAGAGCACAGGTCCTGGCAGCTGCCACAGT CTCCAGGGGCTTTTGCGTTTCTCTCCAGATTTCTAAGGTTAACATGGGGATTAG CTGTTTTGCAATGA	139-364	74	HASSNLLVSRPQCSGNDSAMHR VPGRACVVQCCTSQQEGRGTKE HRSWQLPQSPGAPAFLSRFLRL TWGLAVLQ*
II	cag ATTTCTAAGGTTAACATGGGGATTAGCTGTTTTGCAATGA	325-364	12	FLRLTWGLAVLQ*
III	tag GAAAACAATCATATAA	2342-2357	4	KTII*
IV	tag ATGTGCATCAGTATCTCTGCATCAATATCTCTATATCAGTATCTCTGTGT CAGTGAGCATATGTTGCTGGGCTTAG	2857-2932	24	CASVSLHQYLYISISVSVSICC WA*
V	cag GTCCTAA	3086-3092	1	S*
VI	tag TATGTGTGATTACATTCCTGATTCTGAGCCTTTTTAG	3229-3265	11	MCDYIPDSEPF*
VII	tag ATAG	3266-3269	0	*
VIII	gag TATTTATGACGTGCACAACATTCCTGAATATATTGTCTCTCTCATTTCTC AGATGGGATGTATTGCCTTCTCCATTTCTATTGTTAAAGAAACACTTACAGGGG TTTCTTTAACAACTTGTGAACAGCAGCATCAGAGCCCAGACTACAGCATAAGCA GCTGCTGA	3422-3587	54	IYDVHNIPEYIVSLISQMGCIA FSISIVKETLTGVSLTTCEQQH QSPDYSISSC*
IX	cag ATGGGATGTATTGCCTTCTCCATTTCTATTGTTAAAGAAACACTTACAGG GGTTTCTTTAA	3474-3534	19	WDVLPSPFLLLKKHLQGFL*
X	cag AGTTACCGAGGGCCTCATCAGCGTCAGCAGGAGCCCCTCGCCTTCTGACG CTCTCACATCCTTCTCTCCTGCAGCCCCGTCCTGCCACTGTCCTTGTCCAGCTT CTCTTCAAGGGTCAACTGGTCTACCTTTCCCTACAAGTCTGTCACAGCTTCTTG TTAGCAATCCCTATGGTTGCCCAAAAGCATTTTCAGAGCCTGCATAA	4233-4437	67	VTEGLISVSRSPSPSDALTSFS PAAPSCHCPCPASLQGSTGLPF PTSLSQLLVSNPYGCPKAPSEP A*
XI	cag CCCCGTCCTGCCACTGTCCTTGTCCAGCTTCTCTTCAAGGGTCAACTGGT CTACCTTTCCCTACAAGTCTGTCACAGCTTCTTGTTAG	4307-4394	28	PVLPLSLSSFSSRVNWSTFPYK SVTASC*
XII (Exon 15B)	cag GCCAGGAAATGAGAGTCTCAAAGCCATGTTATTCTGCCTTTTTAAACTAT CATCCTGTAATCAAAGTAATGATGGCAGCGTGTCCCACCAGAGCGGGAGCCCAG CTGCTCAGGAGTCATGCTTAGGATGGATCCCTTCTCTTCTGCCGTCAGAGTTTC AGCTGGGTTGGGGTGGATGCAGCCACCTCCATGCCTGGCCTTCTGCATCTGTGA TCATCACGGCCTCCTCCTGCCACTGA	4870-5107	78	PGNESLKAMLFCLFKLSSCNQS NDGSVSHQSGSPAAQESCLGWI PSLLPSEFQLGWGGCSHLHAWP SASVIITASSCH*
XIII	cag AGTTTCAGCTGGGTTGGGGTGGATGCAGCCACCTCCATGCCTGGCCTTCT GCATCTGTGATCATCACGGCCTCCTCCTGCCACTGAGCCTCATGCCTTCACGTG TCTGTTCCCCCCGCTTTTCCTTTCTGCCACCCCTGCACGTGGGCCGCCAGGTTC CCAAGAGTATCCTACCCATTTCCTTCCTTCCACTCCCTTTGCCAGTGCCTCTCA CCCCAACTAGTAGCTAA	5022-5250	75	VSAGLGWMQPPPCLAFCICDHH GLLLPLSLMPSRVCSPRFSFLP PLHVGRQVPKSILPISFLPLPL PVPLTPTSS*
Exon 16	cag ATGCACTGGGCCAGGTCTTGAAGGCTGTCCAACGAATGG	NA	13	CTGPGLEGCPTNG

FIGURE 4

XIV	cag ACACACTGCCCAGCAAAGGCAAAAGGGCTTCCTTCAACATCAGCTCTGGC CAGTTTGCCAGAGCAAAGCCCTGAGAAAAGCAAGGTTGAAAAGTCTTATTCAAA CTCACCAGGAAAGAGTGGTGTTACTCTCGATGGCGTCTAGCCAGGAATCATGGA ATTATACACCGAGCACCTGTTTGCCATTTTGGATGTTTCCAAACATGAACCAAA CTTCCAGGCCCCTCTGCCATCTCTGGTAA	444-684	79	HTAQQRQKGFLQHQLWPVCQSK ALRKARLKSLIQTHQERVVLLS MASSQESWNYTPSTCLPFWMFP NMNQTSRPLCHLW*
XV	cag TGAGCTGCTAGGACACCCAGCAGAACTTCCCCACTCCACACTGCAATCTC AGGGATCTTAG	849-909	19	ELLGHPAELPHSTLQSQGS*
A431 seq	tag AAGCTACATAGTGTCTCACTTTCCAAGATCATTCTACAAGATGTCAGTGC ACTGA	1633-1687	17	SYIVSHFPRSFYKMSVH*
Exon 17	cag GCCTAAGATCCCGTCCATCGCCACTGGGATGGTGGGGGCCCTCCTCTTGC TGCTGGTGGTGGCCCTGGGGATCGGCCTCTTCATGCGAAGGCGCCACATCGTTC GGAAGCGCACGCTGCGGAGGCTGCTGCAGGACAGGGAG	NA	47	PKIPSIATGMVGALLLLLVVAL GIGLFMRRRHIVRKRTLRRLLQ ERE

FIGURE 4, continued

Co-expression of p170 and p110 EGFR in Chinese Hamster Ovary Cells

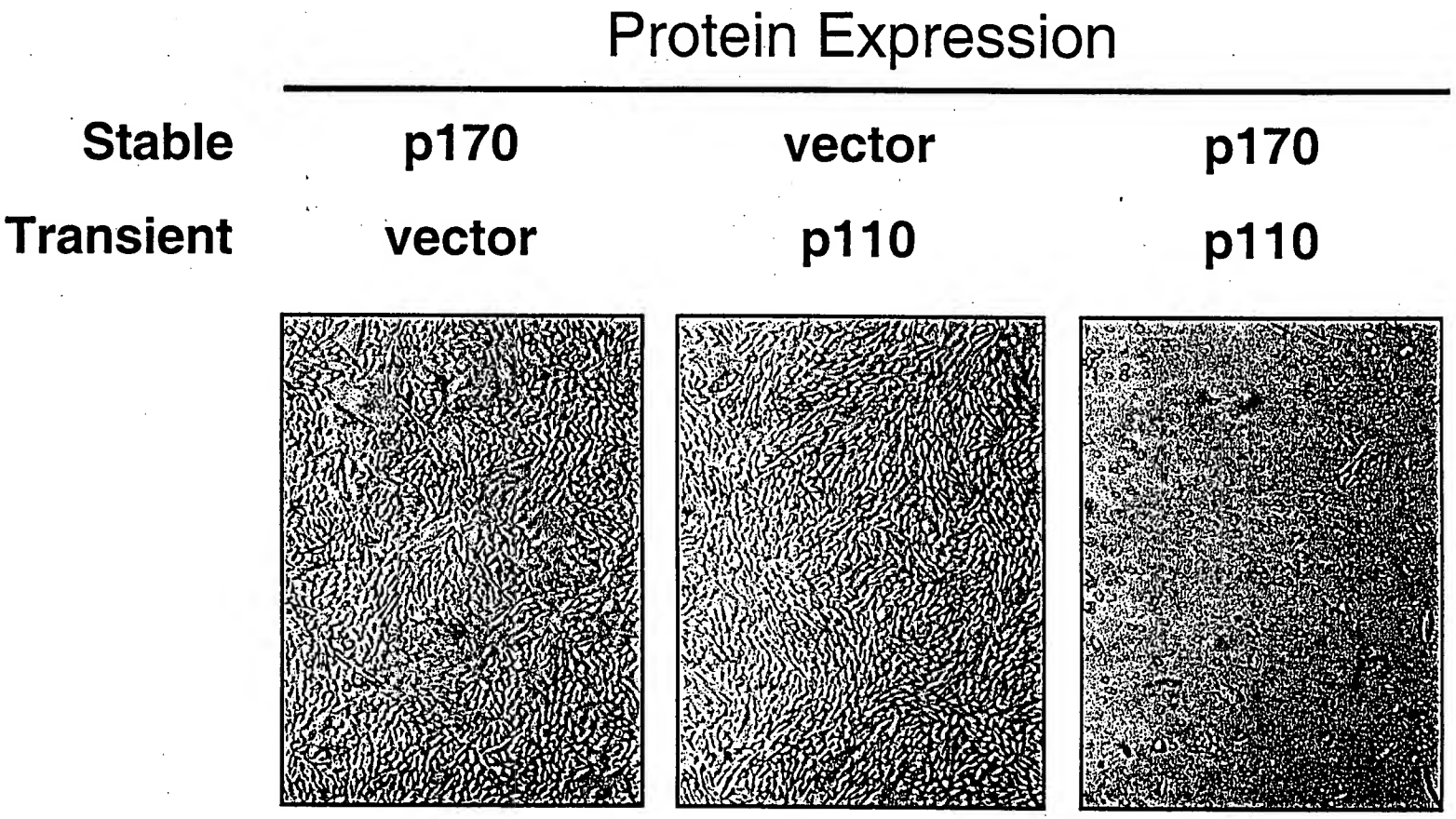
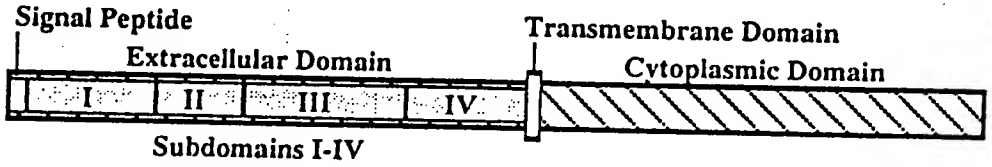


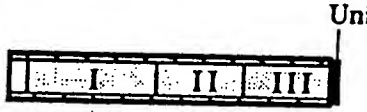
FIGURE 5

A) Full-length ErbB1 Receptor

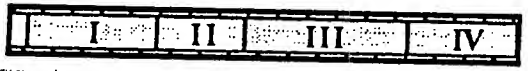


B) Mutant Soluble ErbB1 Analog from Human A431 Cells

C) Soluble ErbB1 Analog from Human Placenta

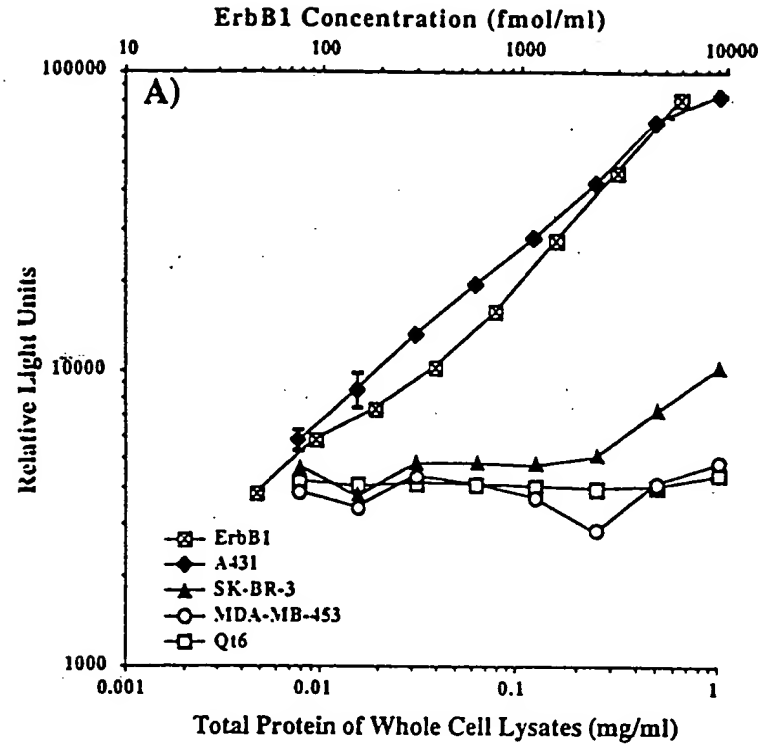


D) Recombinant Soluble Human ErbB1 Analog



mRNA	# of Unique Amino Acids	Molecular Weight	Reference
10.5 & 5.8 kb	none	170 kDa	Ullrich et al., 1984
2.8 kb	17	110 kDa	Ullrich et al., 1984
1.8 kb	2	60 kDa	Reiter and Maihle, 1996
not applicable	none	100 kDa	

FIGURE 6


ErbB1 Concentration (fmol/ml)
10
100
1000
10000
100000
A)
Relative Light Units
10000
1000
0.001
0.01
0.1
1
ErbB1
A431
SK-BR-3
MDA-MB-453
Q16
Total Protein of Whole Cell Lysates (mg/ml)

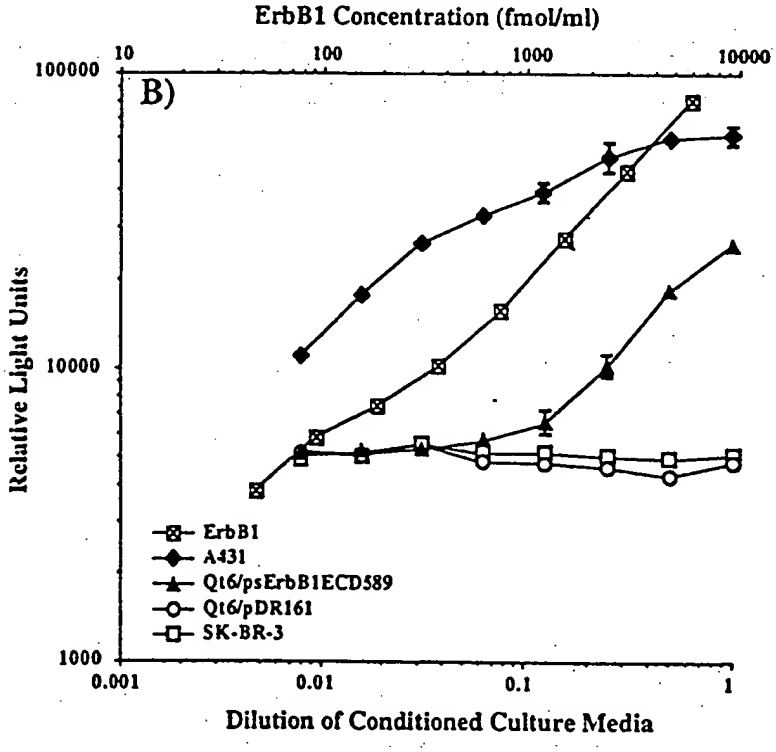

ErbB1 Concentration (fmol/ml)
10
100
1000
10000
100000
B)
Relative Light Units
10000
1000
0.001
0.01
0.1
1
ErbB1
A431
Q16/psErbB1ECD589
Q16/pDR161
SK-BR-3
Dilution of Conditioned Culture Media

FIGURE 7

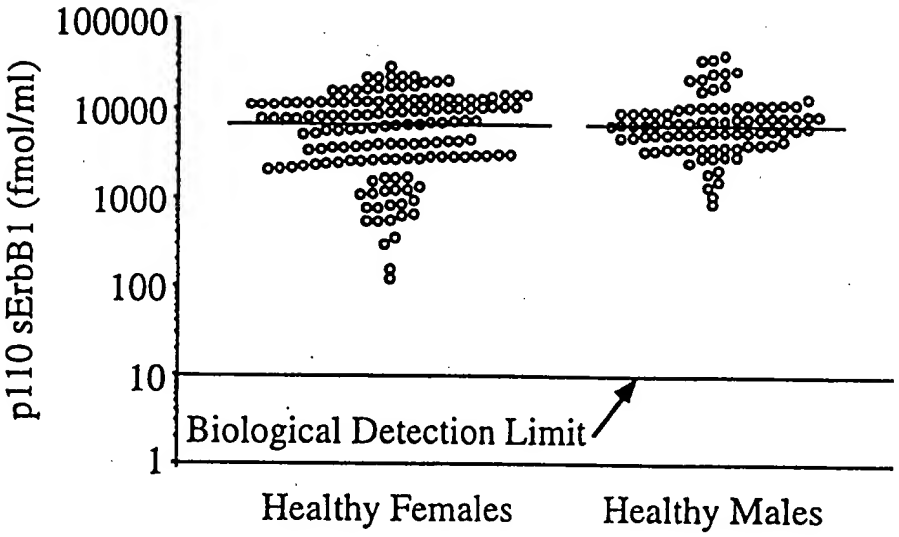

100000
10000
1000
100
10
1
p110 sErbB1 (fmol/ml)
Biological Detection Limit
Healthy Females
Healthy Males

FIGURE 8

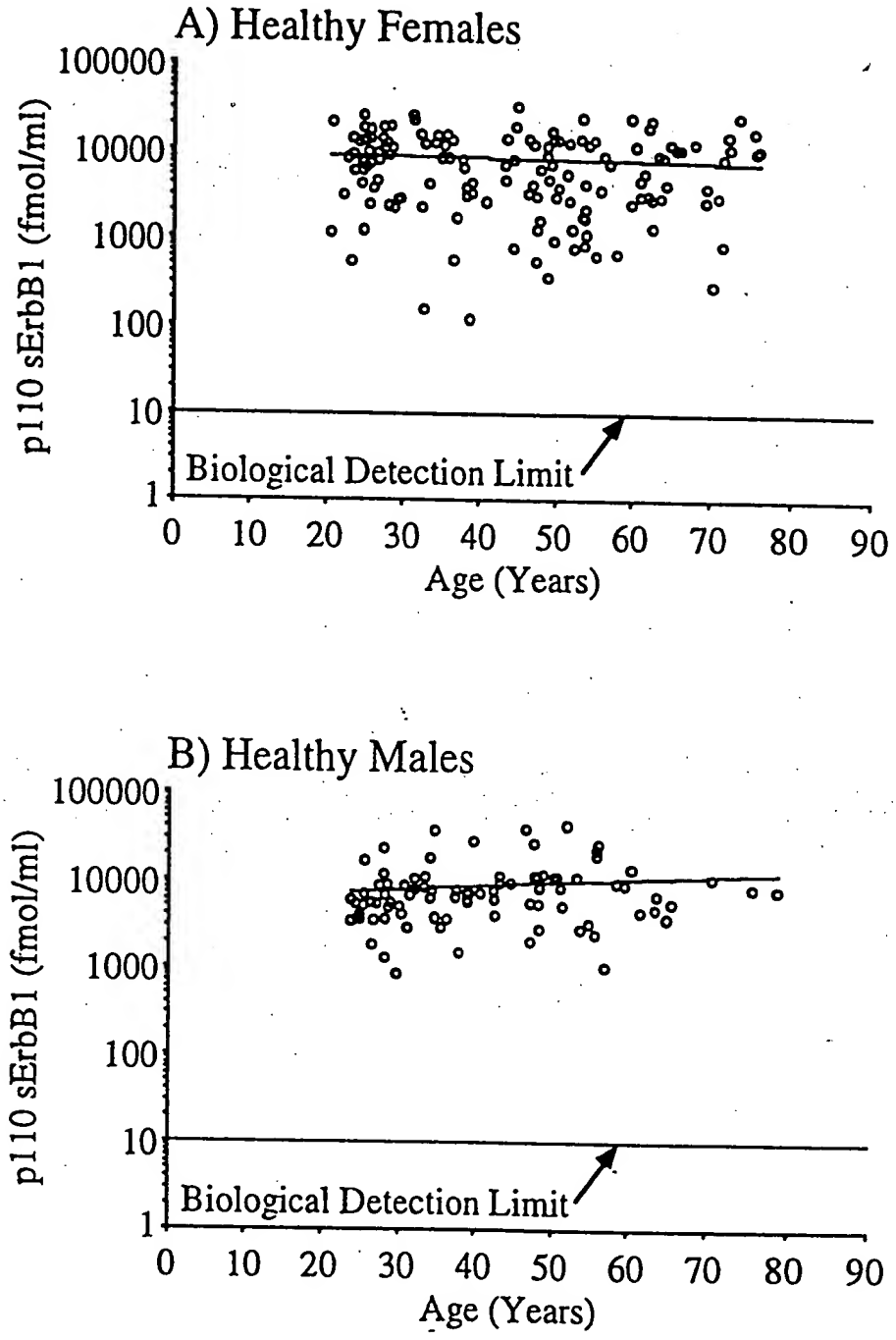

A) Healthy Females
p110 sErbB1 (fmol/ml)
100000
10000
1000
100
10
1
Biological Detection Limit
0 10 20 30 40 50 60 70 80 90
Age (Years)
B) Healthy Males
p110 sErbB1 (fmol/ml)
100000
10000
1000
100
10
1
Biological Detection Limit
0 10 20 30 40 50 60 70 80 90
Age (Years)

FIGURE 9

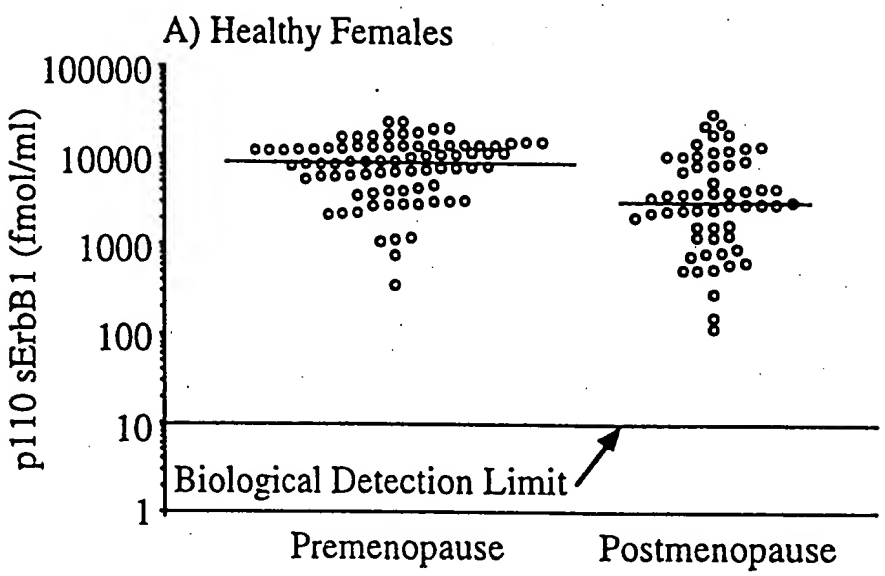

A) Healthy Females
100000
10000
1000
100
10
1
p110 sErbB1 (fmol/ml)
Biological Detection Limit
Premenopause
Postmenopause

FIGURE 10

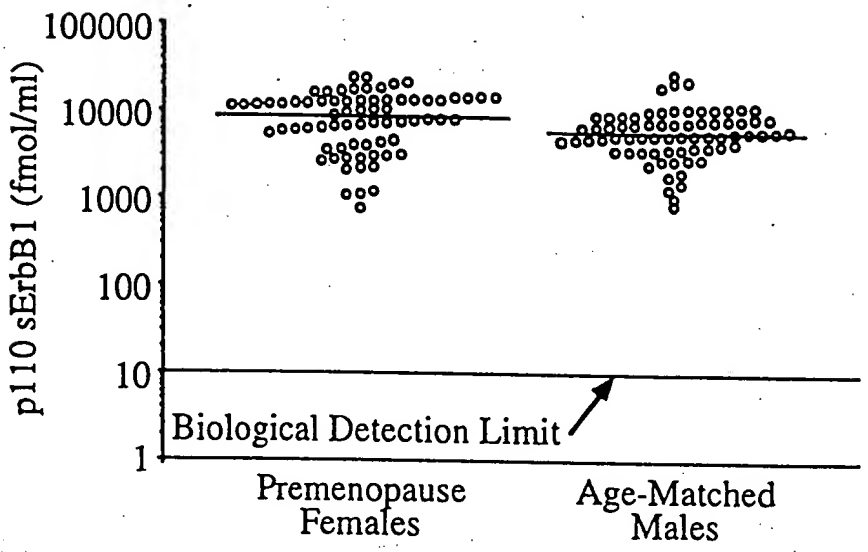
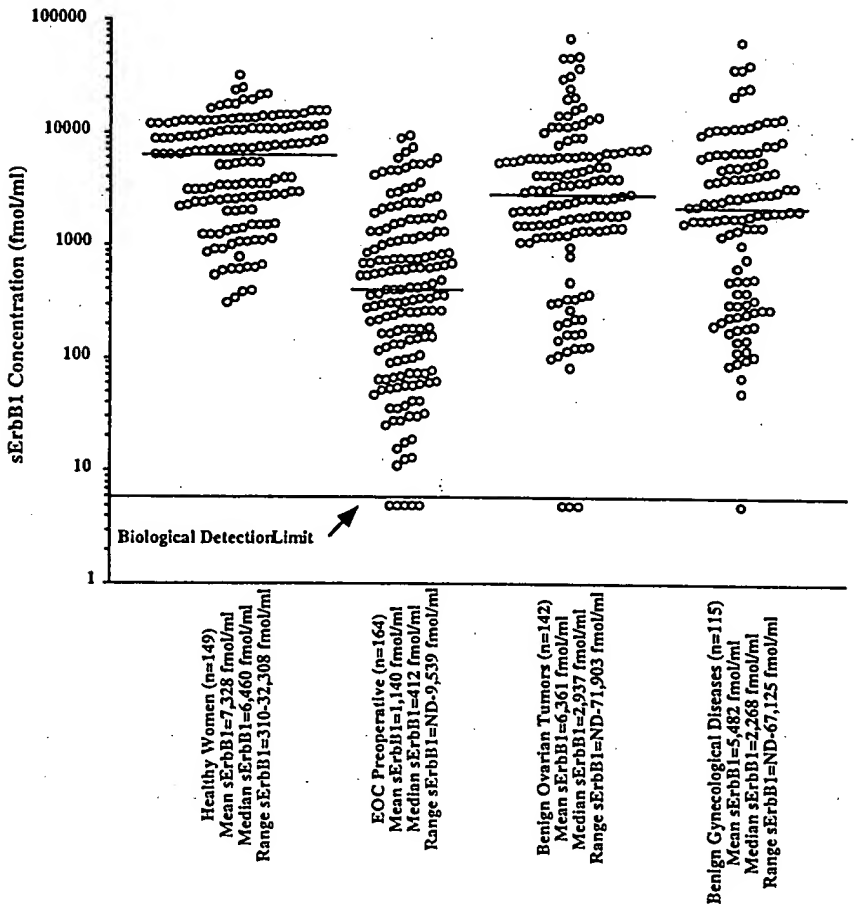


FIGURE 11



Serum sErbB1 levels in healthy women, patients with EOC, benign ovarian tumors, and other benign gynecological diseases as measured by ALISA and compared. Serum samples with sErbB1 levels below the inter-assay biological detection limit (horizontal line with arrow) of 5.89 fmol/ml were arbitrarily assigned values of 5.0 fmol/ml for graphing purposes. Each data point represents the median of the mean sErbB1 concentration for one serum sample tested in duplicate from a minimum of three separate assays. The median sErbB1 concentration for each group of patients is indicated by the horizontal line.

FIGURE 12

FIGURE 13

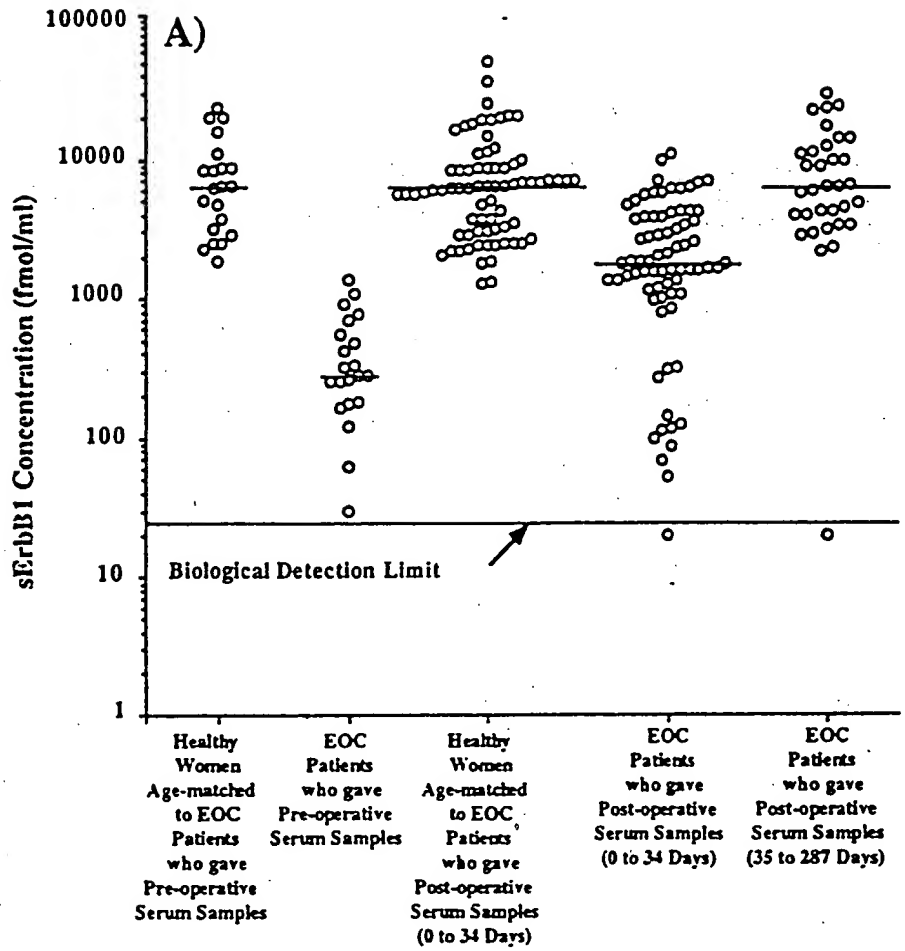

A)
100000
10000
1000
100
10
1
sErbB1 Concentration (fmol/ml)
Biological Detection Limit
Healthy Women Age-matched to EOC Patients who gave Pre-operative Serum Samples
EOC Patients who gave Pre-operative Serum Samples
Healthy Women Age-matched to EOC Patients who gave Post-operative Serum Samples (0 to 34 Days)
EOC Patients who gave Post-operative Serum Samples (0 to 34 Days)
EOC Patients who gave Post-operative Serum Samples (35 to 287 Days)

FIGURE 14

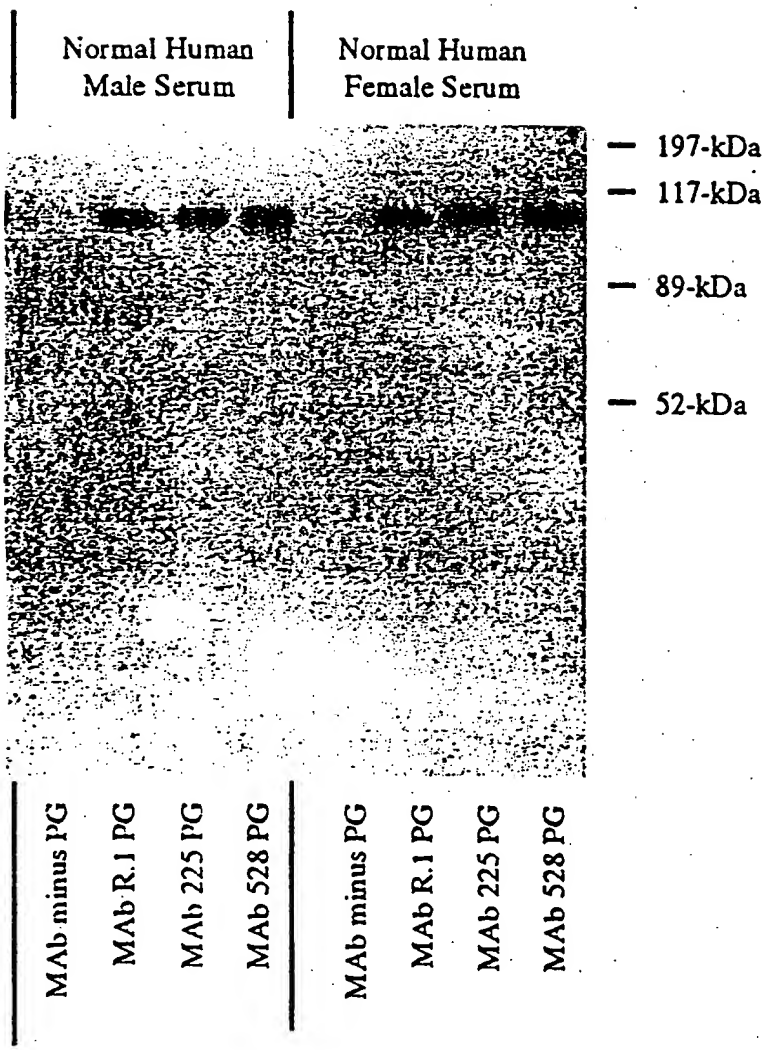
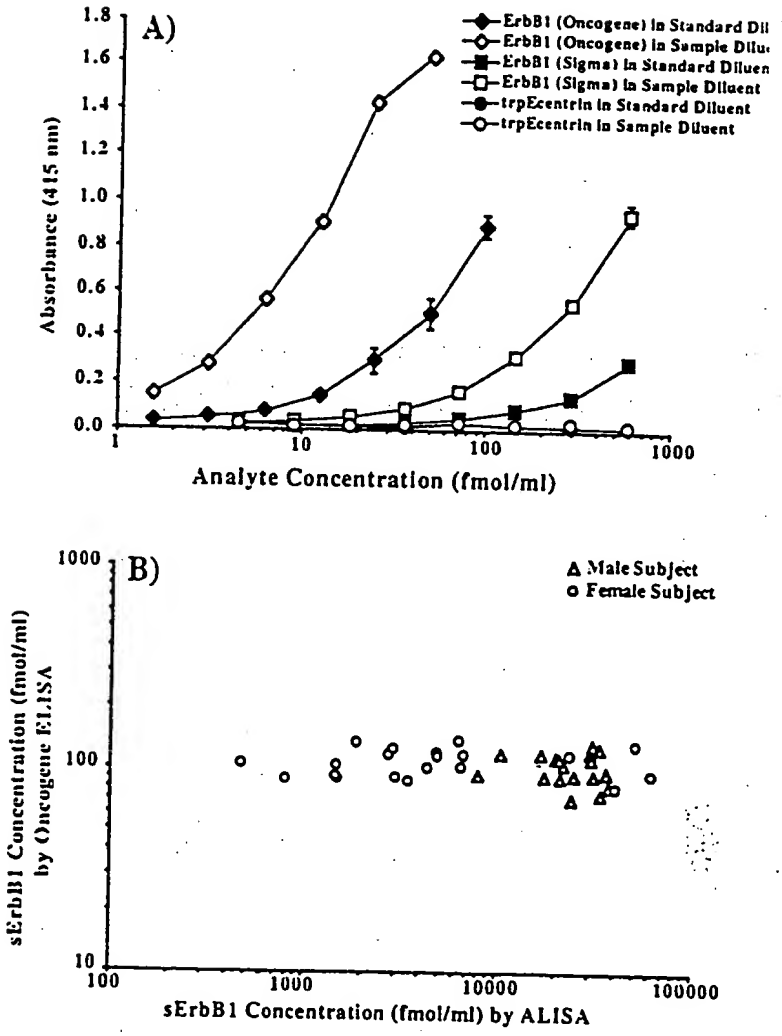

Normal Human Male Serum
Normal Human Female Serum
197-kDa
117-kDa
89-kDa
52-kDa
MAb minus PG
MAb R.1 PG
MAb 225 PG
MAb 528 PG
MAb minus PG
MAb R.1 PG
MAb 225 PG
MAb 528 PG

FIGURE 15


A)
ErbB1 (Oncogene) in Standard Dil
ErbB1 (Oncogene) in Sample Dilu
ErbB1 (Sigma) in Standard Diluen
ErbB1 (Sigma) in Sample Diluent
trpEcentrin in Standard Diluent
trpEcentrin in Sample Diluent
Absorbance (415 nm)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1
10
100
1000
Analyte Concentration (fmol/ml)
B)
Male Subject
Female Subject
sErbB1 Concentration (fmol/ml) by Oncogene ELISA
1000
100
10
100
1000
10000
100000
sErbB1 Concentration (fmol/ml) by ALISA